Harvard Univ

PRELIMINARY DATA

NASA NsG 262-63 Status Report
July 15, 1963-January 15, 1964

RC#1

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SEMI-ANNUAL STATUS REPORT

NASA GRANT NsG 262-63

July 15, 1963 - January 15, 1964

This grant supports interdisciplinary studies of the effects of high energy protons on biologic systems, using the 160 Mev Synchrocyclotron at Harvard University.

During the period 7 days Cyclotron time were devoted to radiation damage studies and 25 days to biomedical studies.

Submitted: _____

Raymond N. Kjellberg, M.D.
Director, BioMedical Laboratory

Approved: _____

William H. Sweet, M.D., D.Sc.
Principal Investigator

_____

William M. Preston, Ph.D.
Co-Principal Investigator

PHYSICS

1. Dosimetry

The detailed mapping of dose distributions from proton beams of various diameters has been progressing satisfactorily. Beams mapped to date are 3, 7, 19, and 32 mm in diameter (example of 7.15 mm beam attached). The mean linear energy transfer (LET) of various depths of penetration has also been measured for the 3 mm and 7 mm beams.

Two types of film that are commonly used in personnel dosimetry, DuPont types 558 and 544, have been calibrated for protons of about 120 Mev and about 13 Mev energy (see Table and graphs). This information was required by Dr. Burke for whole-body irradiation studies.

A detailed assembly of data pertinent to dosimetry and beam monitoring is being prepared in a handbook.

W. M. Preston
A. M. Koehler

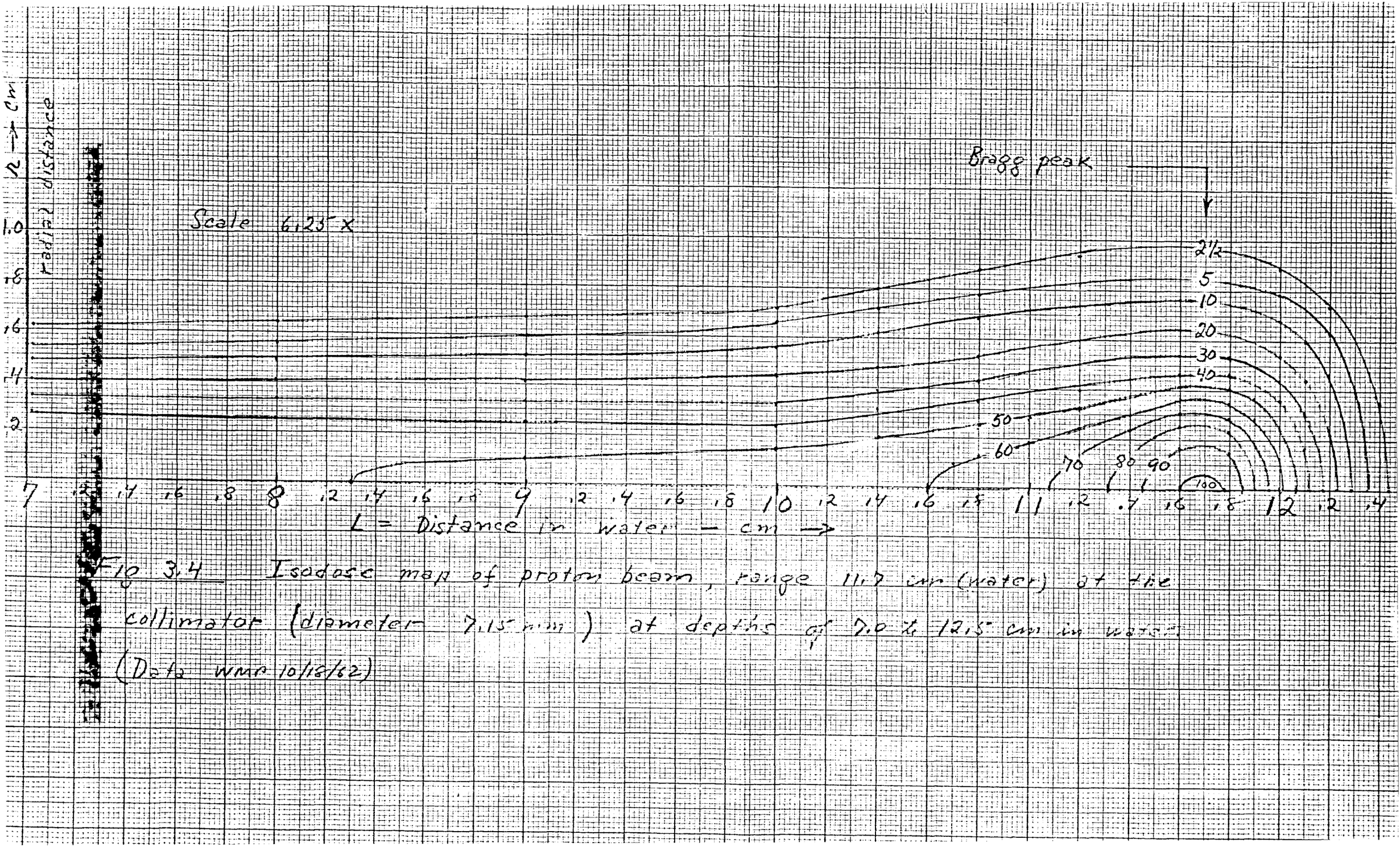
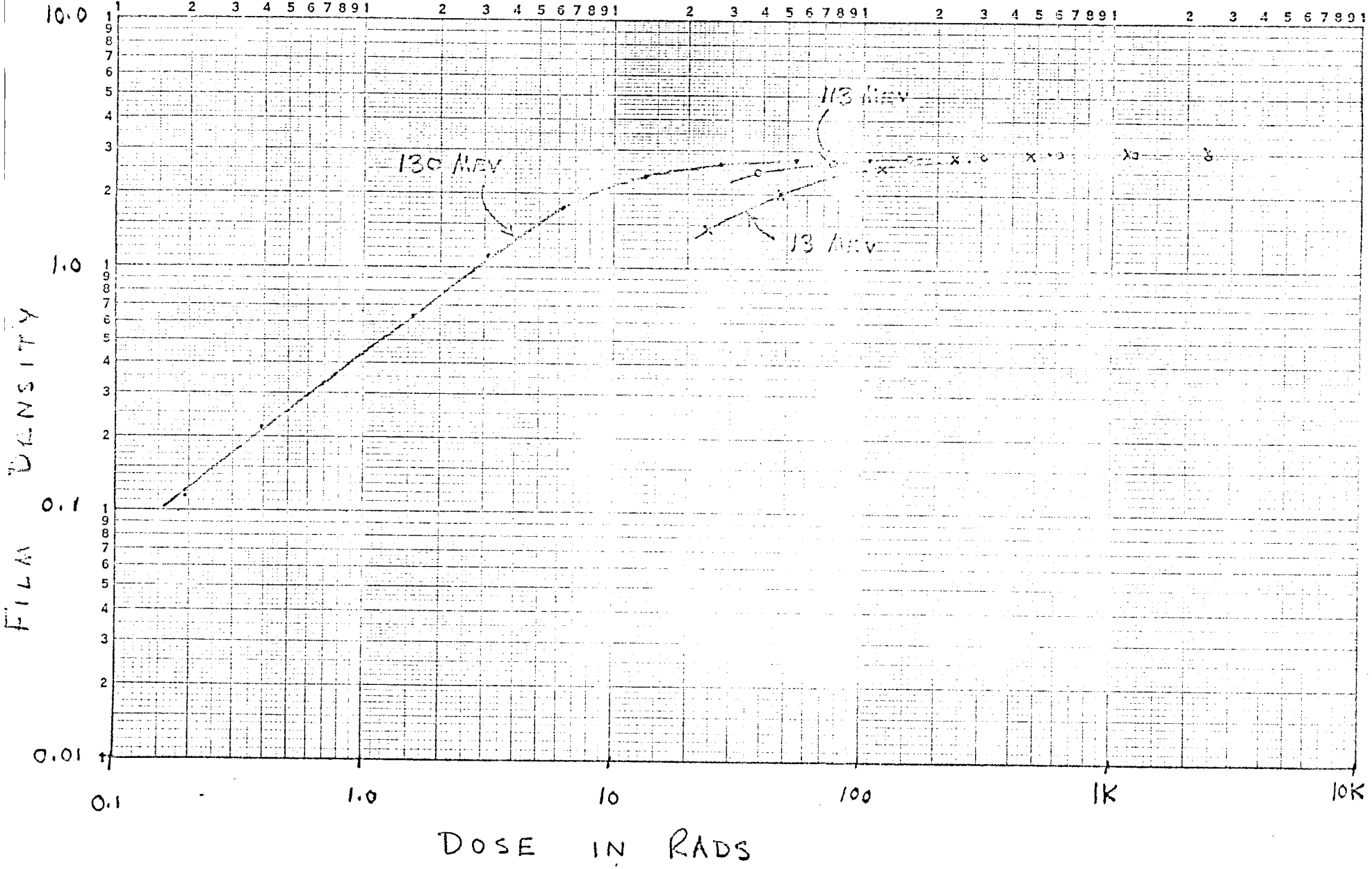


Fig 3.4 Isodose map of proton beam, range 11.7 cm (water) at the collimator (diameter 7.15 mm) at depths of 2.0 & 12.5 cm in water.

(Data WMR 10/15/62)

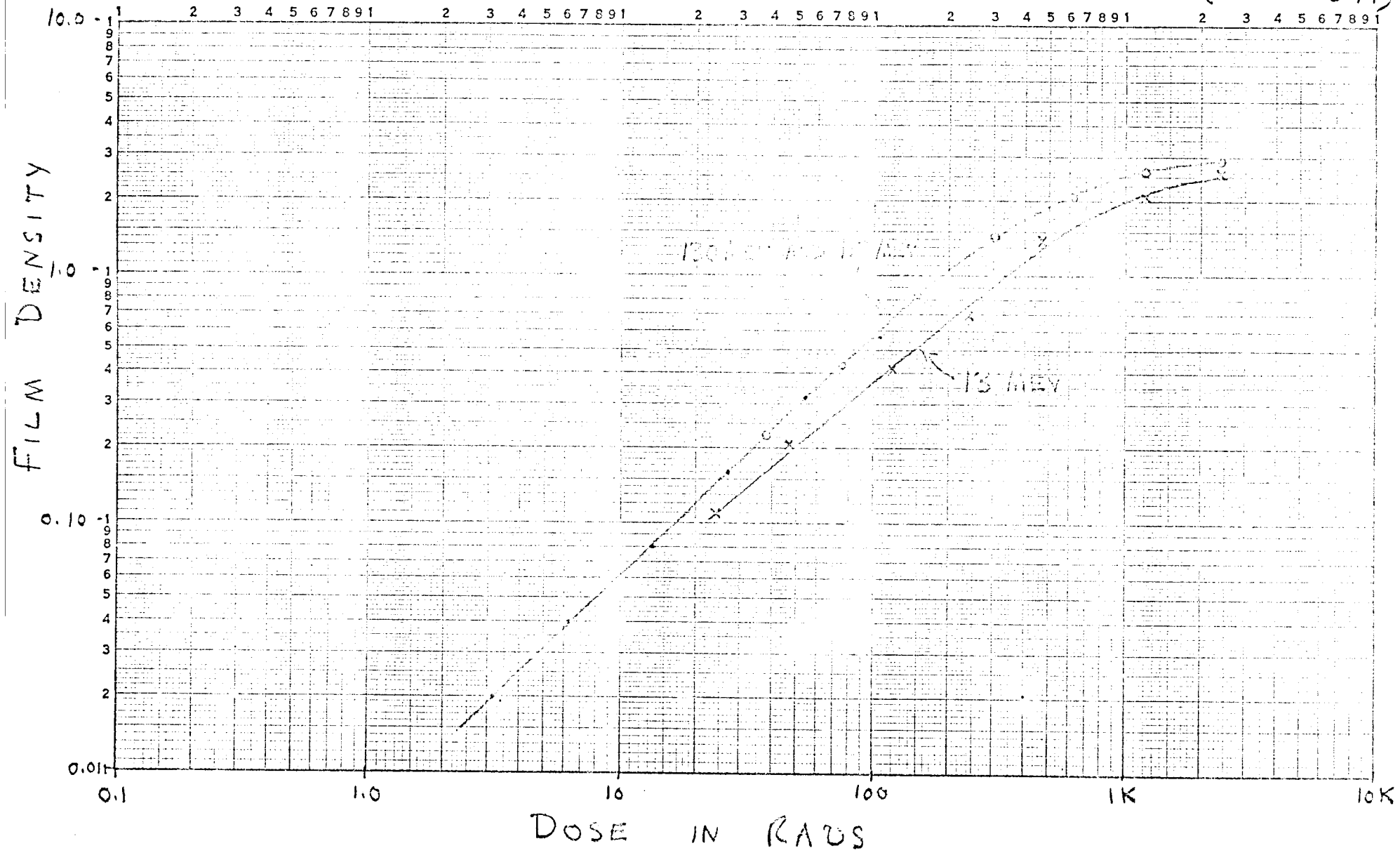
K&E LOGARITHMIC 350-125G
KEUFFEL & ESSER CO. MADE IN U.S.A.
3 X 5 CYCLES

PROTON CALIBRATION - SENSITIVE DOSIMETER FILM (DUPONT 552)



A.M.K. 1 Nov 63

PROTON CALIBRATION - INSENSITIVE DOSIMETER FILM (DUPONT 544)



A.M.K. INV. CO.

Film Dosimeter Calibrations - Summary 30 Oct 63

Date	Energy	Film	Dose	Densities Sensitive	Densities Insensitive	Setup
19●63	~130 Mev	1	0.196 rad	0.120	0.000	0.318" Cu + P18 = 5.61 gm/cm² plexi
		2	0.392	0.220	↓	Col A = 9/32
		3	0.784	0.358		Col B = 1
		4	1.57	0.638	↓	Col C = 1 5/16
		5	3.14	1.122	0.020	Col D = 1 7/16
		6	6.27	1.78	0.040	Calibration via Ch KN_2
		7	13.5	2.35	0.080	using 1 7/16 Col D.
		8	27.1	2.64	0.160	yields 0.196 rads
		9	54.2	2.77	0.320	per monitor count.
		10	108.4	2.80	0.570	
		11	6.27	1.78	0.040	
		12	0.392	0.222	0.000	
		13	0.196	0.115	↓	
		14	0	—		
●Sep63	~113 Mev	1	38	2.480	0.225	Abs P11,12,13,14,16 = 5.832 cm H_2O + water
		2	76	2.740	0.430	Col A = 25/64
		3	152	2.815	0.835	Col B = 1 1/2
		4	304	2.860	1.435	Col C = 1 3/4
		5	608	2.980	2.095	Col D = 2
		6	1216	3.00	2.630	Ch CN_2 (III, 200pf) after B
		7	2432	2.965	2.915	Calibration via diode
		8	0	0	0	yields 3.82 rads / count
		9	0	0	0	
		10	0	0	0	
	~13 Mev	11	23.6	1.475	0.110	Increased water absorber
		12	47.2	2.015	0.205	
		13	118	2.585	0.415	
		14	236	2.850	0.700	
		15	472	2.980	1.400	
		16	1180	3.060	2.070	
		17	2360	3.105	2.605	

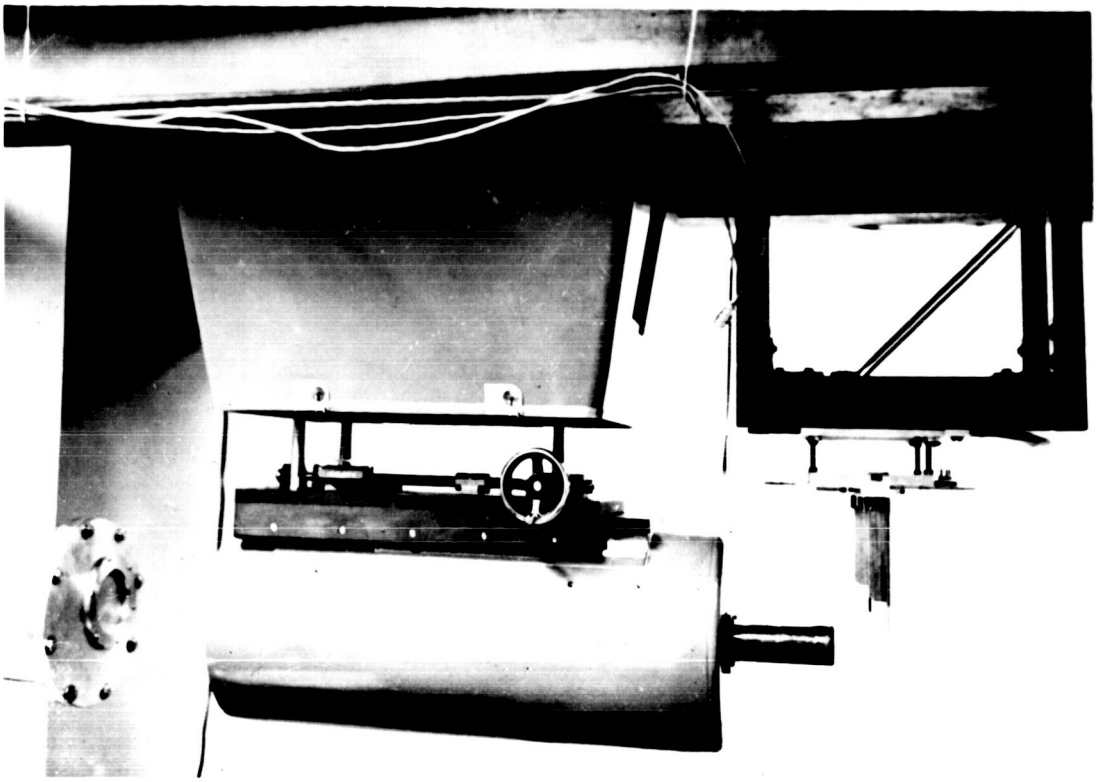
The films are DuPont types 588 and 544

2. Collimation Apparatus

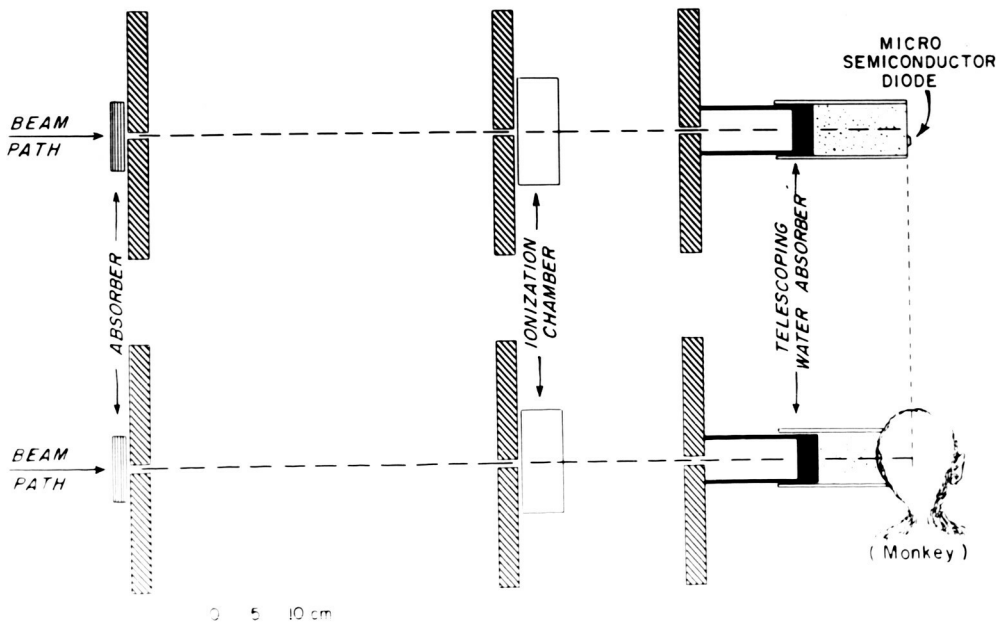
The beam area in the new Biomedical Annex was used for the first time in August. A new set of quadrupole magnets and a system of evacuated beam pipe was installed. Refocussing of the beam in the new area proved more difficult than expected, and we are indebted to Prof. J. N. Palmieri of Oberlin College for his active help in solving this problem.

A new beam-collimator was installed in November. It allows a much greater range of possible beam diameters, and provides better shielding against scattered radiation from the collimator itself. Many mechanical improvements are also incorporated. The following photograph and collimator diagram more fully describe the apparatus.

G. Pollucci
A. M. Koehler



Proton Beam collimator apparatus in Cyclotron Biomedical Building



Function of telescoping water absorber in making in-phantom measurements of dose delivered to living tissue

3. Gamma Ray Absorption Coefficient Measurements in Various Types of Human Tissue

An astronaut, though primarily exposed to particle radiation, will also be exposed to gamma radiation secondaries from shielding and other space environmental sources. The magnitude of this absorption influences the biologic effect. Thus, it is desirable that studies be done to distinguish subtle differences to obtain most critical data of gamma ray absorption in man. The convenient opportunity to do this exists in attempts to delineate tissue abnormalities in the brain of man. Current methods, including positron scanning, lack the necessary accuracy. It is most difficult to delineate the invasive tumors.

A procedure has been developed which in principal is analogous to the positron scanner, i.e. the determination of a variable gamma ray absorption coefficient in an extended medium by measurements made outside the medium. Preliminary measurements were calculated using a spherical lucite model sheathed in aluminum, containing two smaller aluminum spheres. The model cranium corresponds to two space-occupying lesions surrounded by normal brain and a muscle sheath. The reported ratios of the absorption coefficients of lucite and aluminum is 2.3 - roughly the minimum ratio of positron activities in muscle or tumor to normal brain.

Determination of the values of the absorption coefficients was made with the gamma ray beam 5.3 mm wide entering through five portals in one quadrant to the "tumors". 432 observations were made with 20,000 counts each. The agreement between observed and actual values was good.

Positron scanning is complicated by the fact that annihilations along lines joining the counters contribute to observed coincidence rate and must be considered. We think this could be simplified by using isotopes which emit gamma rays closely correlated in time. Cobalt ^{60}, for example, decays to Ni^{60} and emits 2 gamma rays within about 10^{-12} sec.

We plan to continue to investigate the possibilities inherent in the use of gamma rays in scanning with the hope of ultimate high resolution of absorption coefficients in human tissues.

A. M. Cormack, Tufts University

4. 24 to 110 Mev Proton Irradiation of Germanium*

Minority carrier lifetime measurements on n- and p-type germanium were made as a function of proton energy between 24 and 110 Mev. The energy dependence of damage (defect introduction rate) is similar to what has been observed using conductivity and Hall effect measurements to monitor the relative concentration. To repeat briefly, our previous work showed that the energy dependence decreased slower than $\frac{1}{E}$ as calculated using purely Rutherford scattering and the Kinchin-Pease model for the production of secondary displacements. The carrier lifetime studies indicate similar behavior for germanium irradiated with 24-110 Mev protons. Analysis of the lifetime irradiation data using the simple Schockley-Read single level theory does not explain the data, indicating that more than one trapping level is operative in the radiation-induced decrease of the lifetime or recombination rate.

In another series of experiments performed on the cyclotron during the carrier lifetime runs (December 28, 29, 30, 1963), arsenic and antimony doped 10 ohm-cm germanium were exposed to 24 and 110 Mev protons to varying fluxes. Temperature dependence studies of the carrier concentration from $80^{\circ}K$ to $320^{\circ}K$ indicate the presence of only one defect acceptor level 0.24 ev below the bottom of the conduction band. No evidence was found for shallow states ($E < 0.2$ ev) which were expected to result from clustering of defects formed by energetic primaries. These are the conclusions drawn for samples irradiated to $\sim 1 \times 10^{12} p/cm^2$. Further experiments to higher integrated fluxes are planned in the next four month period.

J. C. Corelli, RPI
J. E. Fischer, RPI
J. F. Becker, RPI
A. M. Koehler, Harvard University

* Supported by the National Aeronautics and Space Administration under grant number NsG-290. Proton irradiation and dosimetry provided through grant number NsG-262-63.

BIOMEDICAL

1. The Effect of Low Dosage Long-Term Proton Radiation on the Defense Against Bacterial Invasion

Space travel will expose man to long-term, low dosage total body proton irradiation. To provide suitable defensive and therapeutic measures for protection from his own bacteria and that of his immediate environment, we must first have some knowledge of the effects of this type of radiation upon the host defense mechanisms against bacteria.

This project was begun on July 15, 1963, when an experimental plan, which included the long-term radiation of 60 guinea pigs, was perfected and the necessary equipment for this project (to be carried on at the Harvard Cyclotron) was built. Experiments were carried out to determine the most efficient way to monitor the total dose level achieved over long periods of time at low intensity. It was found that the x-ray film badge method was the most satisfactory.* Irradiation of 60 animals at various low dosages over a period of a week has been carried out. Individual dose levels range from a total of 28 rads to 1500 rads. The level of the various groups of white cells was monitored before, during, and following the radiation and the animals have been sacrificed periodically following the radiation for exhaustive histologic study. In addition, these animals are being studied for the carcinogenic effect of proton radiation with particular reference to renal tissue, using the electron microscope.

The results of the experiment must await completion of the studies indicated, but preliminary survey of the data obtained thus far seems to indicate that the amount of radiation tolerated over a long period at a low dose appears considerably below that tolerated as a single dose given at high intensity. This is a surprising finding and we must carefully check it.

J. F. Burke

* The calibration experiments and all parts of the project relating to actual radiation were carried out by A. M. Koehler (see "Dosimetry").

2. Measurements of Brain Lesions

Biological effects of protons vary significantly in relation to linear energy transfer (LET). At the end of range of protons, as the residual energy falls the LET rises. Careful quantitization of relative biologic effect (RBE) as a function of LET is pertinent to man in space because he will stop within his body nearly all of the protons arising from a solar flare which strike his head, neck, mediastinum, abdomen, pelvis and thighs. This is because the Bragg peak for protons at 160 Mev lies about 17 cm from the surface of a water column, and in tissue it will be a bit less. Only a tiny percentage of the protons from a solar flare have more than 160 Mev energy. We study anatomic effects in the brain of Cebus monkeys to quantitate the relationship between LET and biologic effect.

We have been scrutinizing the size and shape of focal lesions made in monkey brains by means of the protons at various depths of penetration including those at the Bragg peak of ionization. The deviation of the shapes of such lesions from physical isodose plots may be interpreted in terms of an RBE that varies along the path of the protons. Our observations indicate that the ionization per rad is more destructive towards the end of the range, where the LET is higher.

We have attempted to verify this observation by another experiment. Two lesions have been made in a single monkey, using the Bragg peak for one, and using the crossover of higher energy portions of two beams to make the other lesion. In this way, regions of equal dose in rads but with different mean LET can be produced.

The results of analyzing fifteen brains with double lesions, and 17 single lesions as described above, are presented in Table III. The errors quoted are based only on the statistical spread of the data: no satisfactory estimate of systematic errors has yet been made. The experiments yield only values of relative RBE from one part of the proton beam to another. For the moment we have adjusted our arbitrary scale to yield an RBE of unity for an LET of 10 Mev/gm/cm^2, corresponding approximately to the LET of 250 Kvp x-rays. No direct comparison with x-rays has been made.

It should be emphasized that the shape of the lesions could also come about from changes in dimension due to the processing of the brain before microscopic examination. That the shape is not a processing artifact is indicated, however, in the substantial agreement with the conclusion from the crossed-beam experiment. This confirms the gradual increase in RBE for LET near 13 Mev/gm/cm^2 to 40 Mev/gm/cm^2. The extremely high value at 42 Mev/gm/cm^2 is much more doubtful.

A. M. Koehler
C. Hajek

Table III

Adjusted RBE vs. Mean Proton LET

Mean LET		Adjusted RBE[1]
Crossed-beam experiment:		
$\sim$ 13	Mev/gm/cm^2	0.9 $\pm$ .07
Lesion-shape experiments:		
18 $\pm$ .3	Mev/gm/cm^2	0.8 $\pm$ .08
21 $\pm$ .4		1.0 $\pm$ .04
24 $\pm$ .4		1.0 $\pm$ .03
30 $\pm$ .6		1.05 $\pm$ .03
37 $\pm$ .8		1.24 $\pm$ 0.[2]
40 $\pm$ 1.2		1.6 $\pm$ .03
42 $\pm$ 2.		4.1 $\pm$ .22

(1) Scale adjusted to yield 1.0 RBE at approximately 10 Mev/gm/cm^2.

(2) Match-point. All lesions have been matched at one point, yielding zero statistical spread.

3. Neurohistology of Brain Lesions

The study of neuronal cell response to irradiation is vital to any complete evaluation of human response to heavy particle radiation. The cytological changes at varying dose levels will provide useful data in computing tolerance thresholds.

In a previous report we suggested an evaluation of the effect of the proton beam on the basis of the neuronal reaction and its relation to the center of the beam path. Mr. Koehler has measured the lateral spread of the 3 mm beam at several points perpendicular to the major axis of entry. With these figures we have constructed a topographical contour map which we superimpose over similarly enlarged cytological data. We then have, within the individual animal, situations which vary in respect to the total dose but not the rate at which it was delivered.

Our description of a center profile of the beam includes:

1. a measure of the area of central coagulation
2. a measure of the border zone of bizarre cells
3. a measure of the far zone which is a mixture of the dead and living cells
4. these areas are defined by their radiation levels.

We have found that as well as the obvious depopulation of cells in the area of central coagulation, the border and far zone also have a reduced population, which varies with the distance from the path center. Examination for these missing cells has often been overlooked by authors describing the neuropathological effect of high energy radiation.

The far zone has a class of neurons which are dead but which have not been eliminated. These are easily recognized and appear to be disposed along the same continuum as the missing cells. However, at certain levels within the cortex, groups or knots of these dead cells will cluster. It is our intention to examine this grouping closer and perhaps suggest an anatomical basis for their "mass death".

A third type of neuronal reaction to the radiation is a peculiar paling and swelling of the cell body. This particular abnormality is not generally

seen in comparable neuropathological material in the number which we can document. We are following the development of this cell type in our experimental series of increased survival time after radiation. When this phenomenon is properly described, we hope to be able to judge whether this unique reaction is a specific reflection of the high energy particle ionization.

In summary, we are matching the physical profile of the proton beam to the biological profile of the reacting neural tissue and assessing the correlation in terms of the cytoarchitecture of the particular area.

F. R. Ervin
M. Mohnkern

4. Experimental Proton Irradiation in Brain

Prior to irradiation of human beings a large number of animals, usually Cebus monkeys, had been experimentally irradiated. Some of this material has been included in previous progress reports. It has been publically presented on two occasions: in August, 1962 at the Second International Congress of Radiation Research in Harrogate, England and more recently at the Second International Symposium on the Response of the Nervous System to Ionizing Radiation at Los Angeles on August 29, 1963. The Harrogate paper is being published under the auspices of Oak Ridge and the AEC through the offices of Dr. John Kirby-Smith. When reprints are available they will be included with an appropriate progress report. The Los Angeles paper is being published in book form through the offices of Dr. Thomas Haley of the University of California. Because of the excessive volume of these, they are not being reproduced here. However, copies of both papers have been sent to Mr. Leo Fox, RBH, Office of Advanced Research and Technology, NASA, Washington.

The subjects studied in about 150 monkeys given focal brain irradiation with protons at the Bragg peak include:

Mortality and microscopic lesions at doses up to 20,000 rads
Effect of dose rate on mortality
Comparison of effects of high LET at the Bragg peak with lower LET in the path of the proton beam (several methods)
Tolerance of divided large doses of proton radiation
Comparison of divided doses at the same vs. different sites
Acute responses of formed blood elements to high doses of focal brain irradiation.

R. N. Kjellberg

5. Anatomic Changes with Beams of Small Dimension

Tissue response to proton radiation appears to be related to the size of the area irradiated. To date our studies have encompassed principally the use of beams from 3 to 50 mm in diameter. In order to extend the range of these observations in animals and man, we seek to determine thresholds of anatomic change in brain and spinal cord using beams of the smallest dimension currently practical with our apparatus. A knife-like beam of 0.75 mm thickness has been used on the brain and spinal cord of several Cebus monkeys. This study has just commenced and as yet no data have emerged.

R. N. Kjellberg

6. Basic Studies on Biologic Protection Against Proton Radiation

Knowledge of the deleterious effects of radiation on man and animals is largely based on data obtained from x-rays, γ rays and βparticle irradiation studies. Since the radiation hazard to man in space comes almost exclusively from protons, it is imperative to learn whether or not these much heavier particles produce comparable damage, tissue for tissue, to that produced by the same rad doses of x- or γrays. Furthermore, since a man in space may be exposed for occasional short periods to quite high fluxes of proton radiation against which physical shielding would not be practicable, it is important to determine whether radiation mediating agents such as chemical protectors are effective against protons. Such an investigation must include the response of biological systems incorporating such agents to the whole spectrum of proton energies which would be produced during the absorption of a proton of initial energy of the order of 150 Mev in a large biological absorber such as man.

A. Damage at the molecular level.

Since the initial destructive events resulting from interaction of ionizing radiation with any biological system necessarily involve specific molecules, it is fundamental to investigate the disruptive effects at the molecular level. We have chosen two enzymes as representative of proteins as a whole, selecting them on the basis of ready physical assay of the numbers of destructive free radicals produced and parallel biological assay of the radiation destruction of the enzymes' function.

We have irradiated the enzymes ribonuclease and lysozyme and determined the production of long-lived free radicals by electron spin resonance techniques. Summarizing the results to date, we can say that 120 Mev protons and those of much lower energy in the Bragg peak give about 80 per cent as many free radicals as γrays from Cobalt ^{60} under similar experimental conditions. The biological assay figures reveal that 120 Mev protons are 70 per cent, and Bragg peak protons 60 per cent as destructive as Co^{60} γrays to these enzymes when they are irradiated in air at room temperature.

Details of the experiments are as follows:

The samples were packed into thin-walled pyrex capillary tubes, sealed under vacuum and irradiated and stored at 77°K. Care was taken to obtain accurate weights of enzyme in the tubes which were packed in a glove box to exclude moisture. The electron spin resonance spectra of the irradiated enzyme samples were obtained at 108°K, and the relative number of radicals computed for each dose from the first moment of the derivative spectrum and the mass per unit length of the sample. The dose to each sample was computed from the proton beam dose profile curves.

The dose effect curves for both ribonuclease and lysozyme are linear for doses up to 2 megarads, and in this dose range there was no significant difference between the results for "shank" and Bragg "peak" irradiations. The ratio of the sensitivity in terms of free radical production for the two enzymes is ribonuclease/lysozyme = 1.47. A few more irradiations have also been done with higher doses and there are indications of saturation effects and of somewhat larger production of long-lived free radicals by the high energy shank protons compared with the Bragg peak protons. Further experiments are now underway with more accurate estimates of the irradiation doses given in the higher range.

Comparable experiments using Co^{60} γ irradiation indicate that in the dose range up to 2 megarads, the effective RBE value for protons relative to Co^{60} γ is 0.80 ± 0.03 for radical production in these two enzymes at 77°K.

The changes in the ESR spectrum with temperature and the slow decay in the number of radicals remaining at room temperature are being studied. The decay is approximately linear with log t where t is time spent at room temperature after irradiation. The number of radicals remaining falls to about half the initial number detected after 10^5 minutes.

These studies will be extended to other biologically important macromolecules and in addition, the effect of variation in the environmental conditions (temperature, moisture, oxygen, etc.) will be investigated. Such data can throw light on the relative importance of the different classes of radiation damage, only some of which can be modified by external factors. One method of mediating such damage is by the incorporation of certain chemicals and we plan to extend these experiments to determine both quantitatively and qualitatively the modification to the damage produced by proton radiation which is brought about by the presence of protective chemicals such as certain sulfhydryl compounds. It is known that modification in the numbers and types of free radicals produced in x-irradiated enzymes and the corresponding reduction in inactivation

of the enzymes in the presence of such chemical protectors can be determined by the techniques described above. Similar studies with proton radiations of various energies could yield information fundamental to an understanding of the overall effect of the presence of chemical mediating agents in any particular biological target.

B. The effect of radiation-protecting agents upon heavy particle irradiation

The physiologic systems most sensitive to destructive effect of radiation are those in which a relatively large number of cells are in active mitosis. In normal man, the bone marrow cells and cells of the intestinal epithelium are constantly in mitosis and hence especially vulnerable to radiation. A tissue with similar mitotic properties and convenient for experimental purposes is a rapidly growing murine tumor. This is being used to asses response of tissue to proton irradiation and the effect of radiation-protecting agents upon this response. The advantage of this tissue is that its viability following irradiation can be determined by transplantation into a non-irradiated recipient mouse. In this way the response of only one tissue to this radiation may be followed under physiological conditions without the interacting complications which result from the irradiation of large volumes.

To date the tumors of 50 C_3H mice have been irradiated at doses ranging from 2000 to 10,000 rads to determine quantitatively a dose-response curve for these high energy protons. The proton beam with an energy of the order of 100 Mev was used with a 3/4 inch collimator to insure uniform tumor dose. The tumor from each animal was transplanted immediately after irradiation into four recipients and these are currently being followed until definite tumor growth occurs. Normally, growth occurs within 10 days after transplantation but preliminary results indicate that this radiation retards and at high doses prevents growth. These results serve as a baseline from which the effect of radiation-protecting agents (such as aminoethylisothiouronium bromide, AET) in moderating the biological damage of protons may be assessed.

A parallel series of mice are being irradiated with 2 million volt x-rays to correlate the dose-response curves of these two types of radiation. This is

being carried out since the effect of mediating agents upon x-ray damage to tissue is well known, but the effect upon this particular test system has not been previously determined.

This study is at a very preliminary stage. Evaluation of known radiation-protecting agents other than AET, as well as new compounds which we may synthesize, will also be undertaken.

This particular study dovetails with the work of Nystrom and Koehler and their evaluation of cytologic changes by means of electron and microscopic studies.

C. Synthesis and distribution of radiation-protective agents

The effect of high energy protons upon the central nervous system with regard to temporary and permanent alteration in normal function is unknown. However, several recent behavioral studies suggest that deficits in learning and in performance of learned tasks - minor in an experimental animal, but vitally important to an astronaut - occur at relatively low doses. On this basis it would seem pertinent to consider the chemical protection of this vital structure to such particles. Present studies here and elsewhere on the distribution of S^{35} radioactively-labeled protective agents such as AET have shown that the brain attains the lowest concentration of any tissue. Therefore, it appears desirable to synthesize and evaluate protective agents which would be tailor-made for concentration in the central nervous system. Previous work in these laboratories has shown the feasibility of increasing the penetration of certain compounds into the brain by modification of chemical structure with the preservation of biological effect. With this purpose in mind we propose to synthesize compounds containing known active protective moieties with groups which will facilitate its penetration of the brain. These groups, however, must in no way affect the protective action of the compound.

The distribution of these materials in mammals will be determined by labeling them with a radioactive marker, administering them parenterally, sacrificing the animals at intervals and analyzing tissue samples for radioactive

content. It will also be important to determine the period during which protective action occurs.

Compounds which fulfill these two criteria: (1) high concentration in the central nervous system and (2) preservation of protective action will be evaluated completely from a toxicological standpoint.

A. H. Soloway
K. Stratton

7. The Effect of Proton Irradiation on Mitosing Systems

Subcutaneously-grown brain tumors (tumor no. 48) in C_3H mice were irradiated with proton doses ranging from 1000 to 20,000 rads. We then determined the reduction in successful transplantation as a function of dose of protons delivered to the tumors prior to transplantation. Measurements of regrown tumors have been carried out. Compared to the control non-irradiated group, doses in the range of 2000-4000 rads retarded growth one week; doses in the range of 4000-6000 rads retarded growth two weeks; doses above 7000 rads apparently inhibit all tumor growth. However, only five weeks have elapsed since the last experiments; thus regrowth is still possible. Earlier series with follow-up time of 3 1/2 months prove that 10,000 rads inhibits all tumor growth. A total of 104 mice were used for the experiments.

In order to determine cytological changes corresponding to the effects of proton doses delivered to tumors prior to transplantation, specimens of the irradiated tumors were taken for studies of histopathology and fine structure. For this study the material was supplemented with specimens of originally irradiated regrown tumors. Non-irradiated simultaneously transplanted tumors and normal mouse brain were used as a cytological baseline.

The specimens for histopathology have been embedded in celloidin and stained with hematoxylin-eosin, Loyez and cresylviolet stains. The tissue for fine structure studies has been embedded in epon and methacrylate. In the group of tumors irradiated with doses up to 4000 rads no striking immediate changes were observed. Histopathology of regrown tumors was similar to that of the controls. The tumors exposed to 4000-6000 rads showed cells with swollen cytoplasm and shrunken nuclei. Histopathology of regrown tumors in the same group showed some cells of a bizarre shape. In these more slowly growing tumors there were fewer mitoses per visual field than in the control tumors. In the group of tumors irradiated with doses from 7000-20,000 rads there was extravasated fluid around the vessels. The perivascular spaces contained lymphocytes and occasionally erythrocytes. The endothelium of the vessels was partially swollen and showed irregular intracytoplasmic structures. The irradiated normal cerebral tissue showed the same changes.

S. Nystrom
A. M. Koehler

8. Radiation Protection Studies

Various chemical compounds are known to suppress the effects of radiation. To a certain extent radiosuppression of a given compound can be predicted from its molecular structure. Further studies of these structure-activity relationships will add to our theoretical understanding of the mechanism of radiosuppression and permit this means of maximizing protection to an astronaut by development and selection of appropriate chemical compounds.

Several compounds are being tested on mice for preliminary toxicity and for radiosuppressing effects. Preliminary studies indicated that NDGA * exerted a marked radiation protection effect when given in low dosage/mgm/kgm immediately prior to irradiation. These studies using conventional forms of radiation are continuing in preparation for similar experiments on the human tissue explants in the hamster cheek pouch (see following figure) using the Bragg peak of the proton beam.

Dose-Rate Studies

Biologic effects of radiation have been shown to have dose-rate dependence. Further exploration of this dependence is important to anticipating effects of proton radiation to an astronaut, particularly during periods of high flux such as solar flares.

Early studies reported a strong dose-rate dependence for proton irradiation in the monkey brain. In one preliminary study to test the dose-rate dependence in a single system, we utilized both viability and growth of Himalayan barley seeds irradiated with different dose rates. At the same time we used similar doses on the Cobalt 60 source at MIT to give us another indication of the RBE of high energy protons. The data from this study are now being tabulated. In order to make the findings more applicable to man, future studies of dose-rate dependency and RBE will be done in human tissues grown in the hamster cheek pouch, as well as on the entire heads of monkeys and chimpanzees.

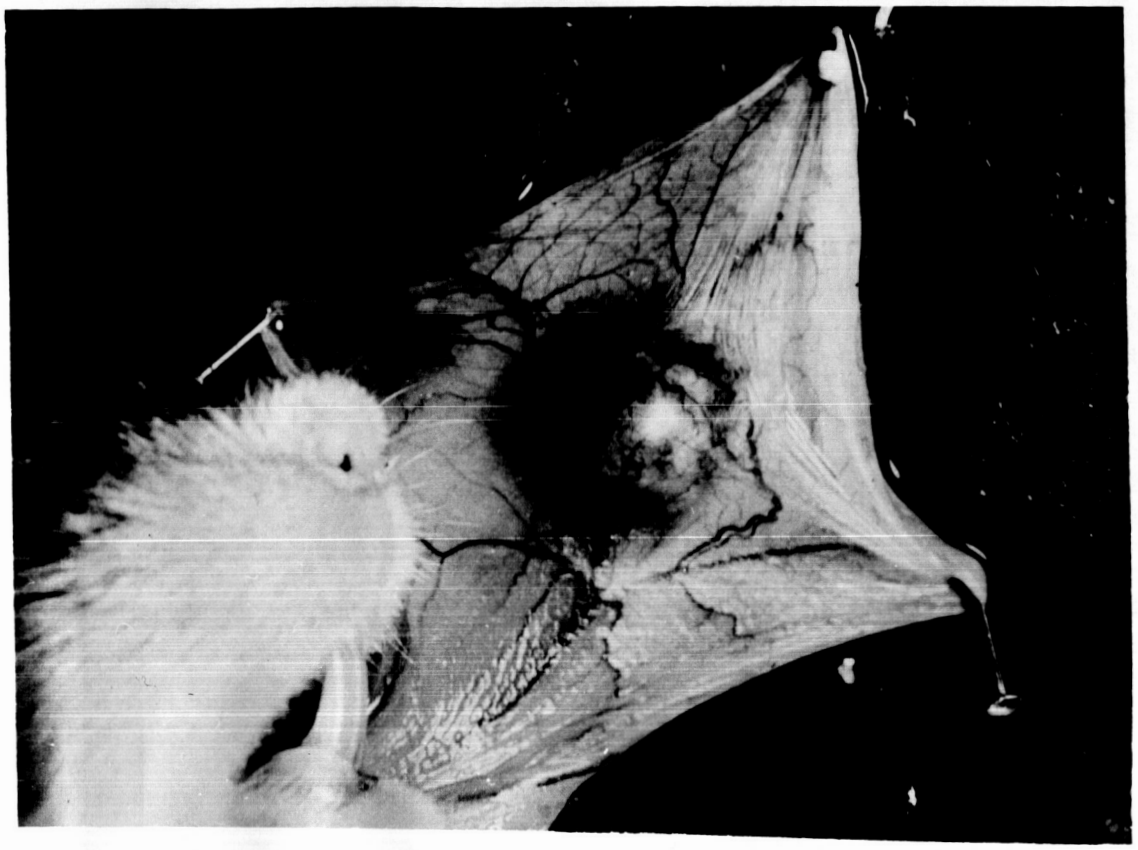
* Nordihydroguariatic acid

Thermoluminescent Bead Dosimetry

Using the Con-Rad TLD dosimeter and readout instrument a comparison was made with existing proton dosimetry by Dr. Preston and Mr. Koehler. Good correlation was obtained for up to 1000 rads. These dosimeters have the virtue of being small and permit readings long after exposure without loss in accuracy. Such a system is ideal for implanting in an animal to get in-vivo dose and because of their small size and negligible weight may be ideal for a space vehicle.

Pulses	Con-Rad TLD System	Harvard Dosimetry
1	530 rads	565 rads
2	1,090 rads	1,130 rads
3	1,480 rads	1,695 rads

C. K. Levy
Boston University



Human adenocarcinoma growing in hamster cheek pouch

9. In-Phantom Dosimetry

An ideal chemical dosimeter for measuring doses of ionizing radiation administered to animals or humans should be a solid or firm substance with the same electron density and Z value as tissue. This absolute rad dosimeter should also show precisely measurable color responses linear with absorbed dose and independent of the radiant energy or its rate of delivery. It should be sensitive to the levels or dose used to expose biologic systems. Such a dosimeter would not only detect radiation but would serve as the phantom; the dosimeter would be the phantom and the phantom itself would be the dosimeter. If the device could be used in the solid state and conveniently sectioned it would make possible an immediate three dimensional color picture of the isodose patterns.

Substitution of fluorine for chlorine and bromine in the paraffin base - halogenated hydrocarbon compound (HAP system) would make an in-phantom dosimeter equivalent or identical to soft tissue in effective Z. The search has continued for a fluorinated compound as the halogenated hydrocarbon in the HAP dosimeter. Many agents have been tried, but none has exhibited the required sensitivity to radiation.

During this period various formulations of three azo dyes and chloroform or bromoform were investigated for their color responses to x-rays and protons. Previously an observation had been made indicating a different color for x-rays than for protons for the same dosimeter formulation. This seemed to be especially noticeable for p-dimethylaminoazobenzene and bromoform which appeared to change from yellow to red with x-rays but showed a response of yellow to brown with protons. There is still a suggestion that this may be the case, but the question has not yet been resolved.

M. S. Potsaid

CLINICAL

1. Proton Radiation of Humans

During the six month period 26 humans were irradiated with protons. In each case, a localized intracranial target was defined and the end of range Bragg peak concentrated in the target zone. The protons enter as beams via a number of portals. The physical parameters of radiation are indicated in Table IV.

A quantity of information is being collected on each irradiated subject. However, a large portion relates to the clinical condition for which the treatment is being done. Summaries of these follow by Kjellberg, Field, et al.

Table V lists responses of subjects which we feel may be related to the radiation per se, independent of the disease process. These observations are pertinent to physiologic responses that may be experienced by man in space.

During radiation, subjects are sedated usually with Nembutal, Demerol, and Trilafon. They may doze intermittently but otherwise are in ready communication with observers. They sit quietly with minor changes in position. They are regularly observed by a neurosurgeon and anesthetist who can observe effects. Blood pressure, pulse, and respiration have been recorded by the anesthetist but we feel that the forthcoming electronic monitoring system will be more critical for scientific purposes.

Following radiation, subjects are transported to the hospital where general observations are made for a varying number of days, after which they return to their homes. Subsequent observations are made by periodic correspondence and visits to clinical observers.

Unanticipated Observations

In the period of long term observation occasionally untoward complications occur. Because of the complexity of the environmental factors influencing humans, it is not possible to exercise the same order of control as in animal experiments, nor to measure precisely the role of radiation in subsequent events.

Three subjects have experienced such untoward events. Since the available material has not yet been fully evaluated, only preliminary notes can be given here.

Subject 1 - W. A. 12 year-old male with a Pontine tumor. Owing to erroneous target delineation a very large dose of proton radiation was given to the anterior pons. A large dose, as much as 18,000 rads had been delivered to certain portions. The patient subsequently died. The pathologic specimen is available, but we await processing and the opportunity to correlate the tissue destruction with the known isodose curves of the radiation.

Subject 2 - C. V. 46 year-old male, was irradiated with 6,000 rads to a pituitary tumor. Subsequently visual deterioration occurred separately in each eye. Surgical (not radiation) considerations weigh heavily in this case, but the possibility of radiation injury exists. We plan to study the available material more thoroughly to aid in reaching a conclusion.

Subject 3 - A. F. 39 year-old female received proton irradiation to the pituitary gland totaling 7,000 rads. Seven months following radiation, blindness evolved in the left eye and swelling occurred in the temporal lobe which had received from 740 to 2,360 rads. The swelling was pathologically proven as due to non-specific ischemic changes and no specific radiation effects were found in the biopsy specimen. A small 2 x 4 mm apparently ischemic zone was seen in the involved optic nerve. While radiation effect is not proven conclusively, it remains strongly suspect. It is surprising that a relatively low dose produced such changes. However, growth hormone is known to produce some unusual responses to radiation. Since this patient had about 10 times the normal amount of growth hormone in circulation this factor may require further assessment.

Pathologic Studies

All of the subjects irradiated with protons have some medical disease which frequently causes the subject's death. We attempt to recover post-mortem specimens when irradiated subjects die. At present we have five

specimens available for study. This critical material takes a long time to evaluate for several reasons. The time course of the patient's disease may be months or years and in most cases is extended by the therapeutic nature of the radiation.

A whole body autopsy is done by a general pathologist at the M. G. H. Subsequently, Dr. Arthur Asbury, a neuropathologist, studies the brain with special emphasis on the pathology of radiation. Although gross observations may be immediately available upon demise, preparation of slides for microscopic analysis and appropriate reporting take many months. When sufficient pathologic data are evaluated we plan to formally report to the NASA and publish the data in an appropriate journal.

R. N. Kjellberg

Table IV

No.	Pt.	Age	Sex	Target Dose (rads)	# of Portals	Target Tissue	Target Size (mm)	Total Irradiation Time (minutes)	Maximum Dose Rate (rads/min)
1	E.O.	67	F	13,900	14	Normal Pituitary	12.0 x 7.1 x 7.1	24.2	658
2	C.D.	50	F	14,140	"	" "	14.2 x 7.1 x 7.1	22.8	803
3	J.S.	60	M	14,154	"	" "	10.2 x 7.1 x 7.1	19.7	871
4	K.M.	63	F	14,000	"	" "	11.2 x 7.1 x 7.1	22.4	1,020
5	H.S.	35	M	14,000	"	" "	11.0 x 7.1 x 7.1	20.3	826
6	L.S.	46	M	13,832	"	" "	16.2 x 7.1 x 7.1	38.4	326
7	J.Y.	42	M	13,832	"	" "	12.6 x 7.1 x 7.1	25.1	581
8	C.W.	50	F	14,000	"	" "	15.4 x 7.1 x 7.1	15.1	1,136
9	E.M.	34	M	12,000	"	" "	17.6 x 7.1 x 7.1	Not complete	545
10	C.F.	35	M	14,100	"	" "	14.0 x 7.1 x 7.1	21.3	753
11	J.M.	44	M	11,844	"	" "	10.6 x 7.1 x 7.1	18.9	919
12	M.O.	59	F	15,140	"	" "	14.0 x 7.1 x 7.1	21.8	756
13	V.G.	32	F	13,860	"	" "	15.8 x 7.1 x 7.1	29.8	572
14	J.L.	44	M	11,900	"	" "	12.0 x 7.1 x 7.1	21.1	594
15	W.M.	45	M	11,914	"	" "	15.0 x 7.1 x 7.1	31.5	393
16	W.C.	40	M	12,000	"	" "	16.0 x 7.1 x 7.1	23.6	564
17	G.G.	57	F	12,000	"	" "	13.0 x 7.1 x 7.1	17.8	861
18	M.R.	50	M	11,900	"	" "	12.4 x 7.1 x 7.1	17.1	726
19	C.S.	75	M	17,000	"	" "	12.8 x 7.1 x 7.1	23.3	713
20	D.S.	50	F	16,800	"	" "	13.8 x 7.1 x 7.1	36.8	662
21	A.A.	66	F	17,100	"	" "	10.8 x 7.1 x 7.1	33.9	544
22	C.M.	13	F	7,902	6	Malignant Tumor of Left Cavernous Sinus	28.0 x 31 x 31	Not complete	735
23	G.Y.	65	F	4,800	16	Pituitary Tumor-Chromophobe	44.2 x 19 x 19	15.3	722
24	M.F.	60	F	5,000	8	Pituitary Tumor-Chromophobe	17.5 x 16 x 16	29.2	857
25	W.L.	45	M	6,060	6	Pituitary Tumor-Eosinophilic	14.8 x 16 x 16	9.1	870
26	M.R.	42	F	14,182	14	Pituitary-Basophil adenoma ?	11.4 x 7.1 x 7.1	19.9	772

Table V

No.	Pt.	0 - 3 days	Over 3 days
1	E.O.	Day 2 - HA, N & V, fatigue, tremor	Day 8 - previous responses improved 2 mo. - hypopituitary
2	C.D.	Day 2 - HA, N & V	6 mo. - continued HA
3	J.S.	No unusual response	1 mo. - hypopituitary
4	K.M.	Not available	2 mo. - continued HA 4 mo. - "sensations" in right side of head
5	H.S.	No unusual response	1 mo. - HA, N & V; radioactive iodine down; insulin reduced; placed on cortisone
6	L.S.	Day 2&3 - HA, V, & anorexia	2 mo. - HA; hypopituitary
7	J.Y.	Day 1 - HA & V	3 mo. - hypopituitism suggested
8	C.W.	Day 1 - HA	Not available
9	E.M.	Day 0 - HA Day 2 - HA, N & V Day 3 - Irritable & agitated	Not available
10	C.F.	Day 1 - HA	Not available
11	J.M.	Day 1 - HA, N & V	Day 4-7 - HA, N & V 3 wks. - HA, N & V continued 3 mo. - protein-bound iodine down; diplopia
12	M.O.	No unusual response	2 mo. - slight HA 3 mo. - continued HA; Left III nerve paresis & ptosis
13	V.G.	Day 0 - Mild HA & N	Day 7-12 - N & V Day 15 - radioactive iodine down Day 18 - protein-bound iodine down Day 20 - placed on corticosteroids 3 wks. - Diabetes Insipidus
14	J.L.	No unusual response	6 wks. - hypopituitism
15	W.M.	No unusual response	Not available
16	W.C.	Day 1 - N Day 2 - HA	Not available
17	G.G.	No unusual response	Not available
18	M.R.	No unusual response	7 wks. - continued HA; subjective hypopituitism; Right III nerve paresis
19	C.S.	No unusual response	Day 17 - hypopituitism 3 mo. - death due to cancer
20	D.S.	Day 1 - N & V related to cancer	Day 17 - continued symptoms, due to cancer
21	A.A.	No unusual response	Day 6 - Onset HA 3 wks. - persistent HA & Diabetes Insipidus 6 wks. - diplopia, left eye
22	C.M.	No unusual response	5 wks. - III nerve paresis; ptosis left eye 3 mos. - proptosis left eye; ? IV nerve involvement
23	G.Y.	No unusual response	5 mo. - diplopia, ptosis, left eye
24	M.F.	No unusual response	No unusual response
25	W.L.	No unusual response	No unusual response
26	M.R.	Day 1&2 - HA related to disease	1 mo. - menses late; HA persists

Abbreviations: HA - Headache
N - Nausea
V - Vomiting

Table V - Summary

0 - 3 days

No unusual response	13
Headache	10
Nausea	6
Vomiting	6
Not available	1
Other	
Fatigue & tremor	1
Anorexia	1
Irritable and agitated	1
Nausea & vomiting unrelated	1
Headache unrelated	1

1 - 6 months

Hypopituitism	11
III nerve paresis and/or diplopia	5
Headache	9
Nausea and vomiting	2
No unusual response	2
Death	1
Unknown	6

1a. Irradiation of Intracranial Tumors

During the period cited four tumor patients (one malignant and three benign pituitary tumors) were irradiated using the Bragg peak of the proton beam. Of the patients treated to date, results are as follows:*

Tumor Classification	# Cases	Early Results (4-5 mos.)	Long Term (> 5 mos.)
Malignant	6	4 improved 2 no change 0 worse	6 deceased
Benign	5	2 improved 3 no change 0 worse	1 improved 1 no change 1 worse 2 too recent
Other	3	2 improved 1 no change 0 worse	1 improved 1 no change 1 deceased
Total	14	8 improved 6 no change 0 worse	2 improved 2 no change 1 worse 7 deceased 2 too recent

* 16 cases treated; follow-up > 1 month on 14 cases

A neuropathologist is studying available post-mortem brain specimens for lesion evaluation. Our current efforts are directed toward altering the size and configuration of the portals to comply with certain findings of the pathologist.

R. N. Kjellberg

1b. Pituitary Ablation for Cancer

Three patients received treatment during the period - one prostate and two breast cancer patients. The cancer patients are receiving a dose of ~17,000 rads to the pituitary.

Post-irradiation results in the ten patients treated prior to January are as follows:

Summary

# Cases	Symptoms	Result
7	Bone pain	6 improved; 1 no evaluation/death
1	Ascitic fluid	1 slightly improved
1	Liver metastases	1 reduction, size of metastases
1	Pain-skin lesions	1 unchanged

Subjectively, results indicate the efficacy of the proton beam pituitary ablation. Our current lack of adequate post-operative endocrine evaluation in these patients precludes an appraisal based on the degree and rate of pituitary suppression as related to the subjective improvement.

Results, however, do encourage further use of the proton beam method of suppressing pituitary function toward relieving pain and possibly slowing the progression of breast and prostate cancer.

R. N. Kjellberg

1c. Pituitary Ablation for Diabetic Retinopathy

During the six month period, 18 patients underwent proton beam pituitary ablation for diabetic retinopathy.

A recent follow-up study of 33 Massachusetts General patients indicates the following results:

Visual Acuity	Endocrine Status
16 improved or the same (57%)	15 decreased 1 no decrease
12 worse (43%)	10 decreased 1 no decrease
5 unknown	6 unknown*

*One patient with decreased vision: no endocrine evaluation

To summarize, although no critical control group is available, the stabilization rate of 57% suggests a beneficial effect and encourages further trials.

Endocrine suppression in those with visual improvement as compared with those showing visual deterioration is not significant.

The order of sensitivity to depression by radiation of the various parameters of assayable pituitary function was: insulin requirement > ACTH > TSH > vasopressin > gonadotropins.

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E. D. U. Powell
W. Reeves
R. N. Kjellberg